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SPEED TALKS

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Abstracts submitted to the 43rd FEBS Congress, taking place in Prague, Czech Republic from 7th to 12th July 2018, and accepted by the Congress Organizing Committee are published in this Supplement of *FEBS Open Bio*. Late-breaking abstracts are not included in this issue.

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SPEED TALKS

Sunday 8 July
17:30–18:30, South Hall 2A

Biochemistry and health

SpT.03-03
New approach for testing inhibitors of HIV-1 uncoating

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Disassembly of HIV-1 core, known as uncoating, is one of the post-entry events essential for retroviral life cycle. The well-balanced stability of the core and participation of host cell proteins ensure proper proceeding of the core disassembly. Uncoating is coupled with the subsequent steps of retroviral life cycle especially reverse transcription and integration of proviral cDNA to the host cell genome. Due to the importance of uncoating, we elaborated and optimized protocol for testing of potential inhibitors of uncoating as promising target for antiretroviral therapy. To compare with the wild type and evaluate the method, we prepared mutations destabilizing (A42D) or stabilizing (E45A) capsid hexameric lattice of the HIV-1 core. The disassembly screening method is based on reverse principle of our previously published method for testing the inhibitors of HIV-1 assembly FAITH. A large set of conditions was tested to determine the optimal treatment resulting in efficient disintegration of HIV-1 core-like particles that were pre-assembled *in vitro* in the presence of tested inhibitors. The results were verified by transmission electron microscopy. To test the efficiency of the best inhibitors resulting from the *in vitro* screening, we prepared expression vectors encoding pseudotyped lentiviruses. Both the wild type and mutant (A42D, E45A) HIV-1 genomes were cloned into the packaging vector psPAX. These were then co-transfected with both VSV-envelope encoding and transfer vectors into HEK 293 cells to test the efficiency of inhibitors added during transfection and infection. To determine whether they target the early if late phase of life cycle, we monitored the time course of reverse transcription. The best non-cytotoxic inhibitors were finally subjected to Cyclosporin washout assay in OMK cells to verify that their target is uncoating. The combination of methods proved to be a useful protocol for screening and verification of substances that inhibit HIV-1 uncoating.

SpT.03-01
Unmasking the impact of methylphenidate on blood-brain barrier function: *in vitro* and animal studies

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Methylphenidate (MPH) is the most frequently prescribed drug for the symptomatic treatment of attention deficit hyperactivity disorder (ADHD), one of the most common neuropsychiatric disorders of childhood. Nonetheless, the central consequences of MPH use are still poorly understood, and its impact on blood-brain barrier (BBB) function has been overlooked. Thus, we used human brain microvascular endothelial cells (HBMVECs), the principal component of the BBB, exposed to MPH (100 μM). Additionally, a rat model of ADHD (SHR) together with the inbred comparator strain (WKY) rats were used and administered for Monday-Friday with vehicle or MPH (1.5 or 5 mg/kg/day, per os) from P28 to P55. Using FRET-based live cell imaging, together with pharmacological inhibitors and lentiviral-mediated shRNA knockdown, we revealed that MPH promotes ROS generation via activation of Rac1-dependent NADPH oxidase and c-Src activation, which then phosphorylates caveolin-1 (Cav1). Moreover, Cav1 knockdown in HBMVECs prevented the increased permeability and leukocytes transmigration triggered by MPH. In accordance with cell results, MPH (5 mg/kg/day) induced cortical BBB permeability in both WKY and SHR animals. However, microvascular alterations were more prominent in WKY characterized by a marked disruption of intercellular junctions, an increase of endothelial vesicles, and an upregulation of adhesion molecules concomitant with peripheral immune cells infiltration. Furthermore, MPH promoted a robust neuroinflammatory and oxidative response in control rats. Curiously, the lower dose of MPH (1.5 mg/kg/day) had a beneficial effect since it balanced both immunity and oxidative status in the ADHD model. Overall, our results show that MPH can led to brain vascular alterations particularly under physiological conditions. This highlights the importance of an appropriate MPH dose regimen for ADHD, and also that MPH misuse can have a negative impact in the brain.

SpT.03-02
Alterations in mitochondrial multimeric dehydrogenase complexes in a cardiac model of Friedreich ataxia

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Friedreich ataxia is a neurodegenerative and inherited disease caused by a decrease on frataxin protein expression. Frataxin is a mitochondrial protein that has been related to iron homeostasis,

oxidative stress and energy metabolism. Heart is one of the most affected organs in this disease. We have set up a cellular model of neonatal rat ventricular myocytes (NRVM) where frataxin is down-regulated by interference RNA. The main phenotypes present in this model are mitochondrial network disorganization with enlarged mitochondria, lipid droplets accumulation and oxidative stress increase. We have investigated the presence of proteins with thiol-redox state alterations by two dimensional electrophoresis. Three proteins were identified: Electron transfer flavoprotein-ubiquinone oxidoreductase (ETFD), Dihydrolipoyl dehydrogenase (DLDH) and ATP synthase subunit alpha (ATPA). As DLDH is the E3 component from Pyruvate dehydrogenase (PDH) and α-ketoglutarate dehydrogenase (αKGDH) complexes, we decided to measure the content of two subunits from the E1 and E2 components of these complexes by SRM-based targeted proteomics. We also measured E2-bound lipoic acid by western blot. Overall, these results indicate that in frataxin-deficient NRVM there is a significant loss of the E1 components from PDH and αKGDH complexes. Total protein-bound lipoic acid levels were not altered in frataxin-deficient cardiomyocytes, but their redox status was compromised as it was found in a more oxidized form. We also analyzed the presence of glutathionylated proteins and we found that actin is glutathionylated in frataxin-deficient cells. To sum up, frataxin-deficient cardiomyocytes present an altered thiol-redox state that could impair mitochondrial metabolism and contribute to the cardiac pathology. Thiol-containing antioxidants and PDH cofactors are being investigated as potential therapeutic approaches to ameliorate the cardiac pathology of Friedreich Ataxia.

SpT.03-10
Mito-cytoplasmic translational balance in the regulation of longevity

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Aging has long been considered to be a passive process. However, mounting evidence shows metabolic- and nutrient-sensing pathways contributing strongly to its plasticity in a manner affecting health and longevity. We recently demonstrated that inhibition of mitochondrial protein translation acts as a conserved longevity mechanism that extends lifespan. This was found to be critically dependent on the mitochondrial unfolded protein response, a mechanism that is conserved from nematodes to mammals. Remarkably, treatment with Rapamycin, a model for both decreased cytosolic translation, and which by itself is a classic mechanism for increasing lifespan, was also found to induce the mitochondrial unfolded protein response, suggesting cross talk between these two longevity pathways. In line with this, we have found that inhibiting mitochondrial translation initiates a signaling cascade leading to a coordinated repression of cytoplasmic translation. We term this *mito-cytoplasmic translational balance*, and propose it to be a mechanism that can be employed to promote healthy aging. To explore the link between mitochondrial and cytoplasmic translation, we have used a worm model of impaired mitochondrial translation (RNAi mediated knockdown of *mrps-5*). By integrating 'omics' data on three levels of biology; transcriptomics of the whole worm, transcriptomics of the polysomal fraction of the worm (highly translated transcripts), and proteomics, we present an integrated, system-level view of the molecular changes induced via knockdown of mitochondrial translation that result in longevity. Furthermore, we show key elements of this process to be conserved when looking at mouse liver tissues and human K562 cells, using the antibiotic doxycycline as a means to reduce mitochondrial translation. These findings indicate a strong potential to target this pathway in humans to promote healthy aging.

SpT.03-07
Recombinant lactic acid bacteria with chemokine-binding and antioxidant activity for the treatment of inflammatory bowel disease

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Probiotic lactic acid bacteria can be engineered to secrete or display on their surface recombinant proteins that decrease inflammation. Consequentially, they can serve as oral delivery vehicles that can improve the symptoms of inflammatory bowel disease (IBD). Tick chemokine binding proteins evasins have the ability to bind and neutralize different chemokines and inhibit the chemokine-mediated recruitment of leukocytes. Three evasin genes were cloned into surface display vector and over-expressed in *L. lactis* NZ9000 and NZ9000*ΔhtrA* in fusion with secretion signal and surface anchor. Evasin-displaying bacteria removed 15% to 90% of 11 different chemokines from the solution. The binding was dependent on bacterial concentration. Evasin-3 displaying *L. lactis* NZ9000*ΔhtrA* cells had superior chemokine binding ability and removed 88.0% of IL-1β-induced CXCL8 from the supernatant of Caco-2 epithelial cells. The gastric stable pentadecapeptide BPC-157 is able to prevent and treat gastrointestinal inflammation by reducing the production of reactive oxygen species (ROS). We engineered *Lactococcus lactis* as a vector for the delivery of BPC-157 by anchor-mediated surface display and trypsin shedding, or by secretion to the growth medium. BPC-157 release from the bacterial surface or from isolated fusion proteins by trypsinization was demonstrated with novel anti-BPC-157 antibodies or mass spectrometry. The concentration of BPC-157 delivered by surface display of BPC-157-fusion protein and trypsinization amounted to 30 ng/ml. It increased to 117 ng/ml by USP45 secretion signal-mediated secretion, making the latter the most efficient lactococcal delivery approach for BPC-157. Secreted BPC-157 significantly decreased ROS production in 149BR fibroblast cell model as determined by flow cytometry. Evasin-displaying and BPC157-producing probiotic bacteria were effective in cell models and are highly promising as innovative supportive treatment of IBD.

SpT.03-12
Reconstructed antibodies from domain swapping for cancer therapy

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Antibody is a protein which has been fragmented and rearranged to form recombinant proteins with non-native structure and function, because antibody is a typical module protein that is composed of structurally and functionally independent fragments. In this study, we propose a new structural format of high cytotoxic bispecific antibody assembly recruiting to cancer and lymphocyte cells. Bispecific antibodies have been used to induce synergistic signals in the cells by forming linkages between the two target antigens on cell surfaces. Cross-linking of immune cells with cancer cells induces the immune cells to damage the cancer cells;

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especially, highly cytotoxic T cells, which are not activated by natural antibodies because T cells have no Fcr receptors, are targeted for recombinant reconstruction of homogenous bispecific antibodies. Here, we constructed a compact bispecific and bivalent antibody from antigen-binding modules of single variable domain of the heavy chain of a heavy chain camel antibody (VHH) and single chain Fv (scFv). The single domain format of VHH is an appropriate building block for generating fusion proteins and the scFv has the potential of intermolecular interaction that induces dimerization: the VHH domains with affinity for the epidermal growth factor receptor (EGFR) overexpressed on cancer cells were fused to the self-dimerized scFv recruiting to CD3 receptors on T-cells, resulting in the formation of bispecific and bivalent antibodies (BiBian). The VHH-fused scFv fragments were spontaneously assembled to BiBian forms with a unique seahorse conformation in bacterial expression, and affinity increment for both target cells by bispecific and bivalent design of BiBian caused a drastic enhancement of cytotoxicity against tumor spheroids *in vitro* and *in vivo*. We show a promising seahorse-shaped high cytotoxic antibody assembly formed from small antibody modules expressed in bacterial expression.

SpT.03-09
The fibrogenic activation kinetics of stromal stem cells in three different skeletal muscle degeneration models

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Chronic skeletal muscle degeneration is characterized by fiber atrophy and deposition of extracellular matrix components. Accumulation of ECM leads to irreversible fibrosis. Recent studies are focused on a stromal cell population named fibro-adipogenic precursors (FAPS) that are primarily responsible for the development of fibrosis. Although precise molecular markers of FAPs are lacking, during acute injury repair, FAPs are known to be regulated though two main inflammatory cytokines TNFa and TGFbeta and immunophenotyping using $CD140a^+$, $Sca1^+$, $CD31^-$, $CD45^-$, $CD11b^-$ was shown to be adequate. However, fibrosis is also accompanying disuse atrophy and sarcopenia without any inflammatory background. It is not known whether if or how FAPs are involved in fibrosis without inflammation. The aim of our study is to investigate the involvement and activation of FAPS in two different models mimicking different stromal components of chronic muscle degeneration. Tenotomy immobilization and denervation induce endomysial fibrosis and fatty infiltration respectively without provoking inflammation. Immobilization models are employed on 8–12 weeks male Swiss mice receiving BrdU and acute muscle injury is used as a positive control. The mononuclear cell population was isolated and FAP population was enumerated and BrdU incorporation was assessed after seven days of tenotomy and ten days of denervation using flow cytometry. Results confirmed activation and proliferation of FAPs in both tenotomy and denervation without any evidence for inflammation. These results pinpoint the fact that inflammation is not a pre-requisite for FAP activation and fibrosis development in skeletal muscle. The mechanistic activation basis of latent TGF-beta without inflammation, are not known.

SpT.03-08
Analysis of human serum glycoproteins by native chromatography and hybrid mass spectrometry approaches

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Many proteins in human blood serum show complex combinations of post-translational modifications (PTMs). These modifications affect the functionality and clearance of the product. Serum glycoproteins also represent some of the most relevant protein biomarkers. Next to changes in abundance, changes in PTM/glycosylation profiles may provide even stronger biomarker signatures. Human blood serum-derived acute phase proteins were purified by native chromatographic separation directly from human serum and subjected to detailed structural analysis by combining two advanced mass spectrometry-based methods; high-resolution native mass spectrometry (MS) and peptide-centric MS. Firstly, a panoramic view of the intact glycoproteins was acquired on a modified Exactive Plus Orbitrap instrument with extended mass range. Subsequent peptide-centric analysis of the (glyco)peptides provided site-specific quantitative profiles of all PTMs. In this way, we analyzed human fetuin, α-1-antitrypsin and hemopexin. In addition to the acute phase proteins, we also examined various components of the complement system and demonstrated a presence of new PTMs. Furthermore, we introduce a novel algorithm that allows a direct comparison of the data obtained from the two independent approaches (native MS and peptide-centric proteomics). This algorithm can be efficiently used for defining biosimilarity between proteins, which would be beneficial for the stratification of therapeutic proteins. Our data reveal new insights into the structural heterogeneity of biologically important serum glycoproteins, exposing new PTMs and sequence variants. We in detail revealed PTM localizations, relative abundances and glycan structures in a site-specific manner. Therefore, we conclude that our presented combined approach uniting native mass spectrometry and peptide-centric proteomics for the structural analysis of glycoproteins may find applicability in biotherapeutics as well as biotechnology.

SpT.03-06
Targeting nanoparticles to modulate mTOR activity in cancer cells

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Cancer is a major public health problem worldwide and is the second leading cause of death in the world. In recent years, there has been an unprecedented expansion in the field of nanomedicine with the development of new nanoparticles for the diagnosis and treatment of cancer. Yet, we still possess incomplete understanding of nano–bio interactions on cellular and molecular levels. Such gap in the knowledge creates hurdles for clinical translation and commercialization of nanomedicines. Here we demonstrate that amino-functionalized polystyrene nanoparticles trigger cell death in hepatocellular carcinoma cells. We decoupled molecular mechanisms of nanoparticles action on cancer cells and used specifically functionalized nanoparticles as anti-cancer drugs that can bypass a tumour cell's ability to develop drug

resistance. We demonstrate that amino-functionalized polystyrene nanoparticles, but not amino- or hydroxyl-functionalized silica particles, trigger cell death in hepatocellular carcinoma cells. Importantly, biodegradability of nanoparticles plays a crucial role in regulation of essential cellular processes. We propose the following model of different nanoparticles action on proliferation liver tumour cells. Silica nanoparticles are rapidly internalized by the studied cells with subsequent localization to lysosomes. Due to inherited instability, silica nanoparticles are rapidly degraded by lysosomal content. This degradation results in mTOR activation. Activation of the mTOR pathway contributes to cell proliferation. Contrary, polystyrene treated cells exhibit proton accumulation in lysosomes associated with lysosomal destabilization and damage of the mitochondrial membrane. At the molecular level, polystyrene nanoparticles obstruct mTOR signalling leading to cell death. Our data provide fundamental knowledge which could help in developing safe and efficient nano-therapeutics.

SpT.03-04
The lesson of Russian part of human proteome project: transcriptome and proteome encoded by chromosome 18

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The aim of the International Human Proteome Project is to study products of gene expression using a chromosome-centric approach. According to this approach, each country participating in the project focused on the proteins encoded by a specific chromosome. Russian team chose to study the size of the proteome, which depends upon the number of different proteoforms (*width*) and the number of copies of an individual proteoform in a biosample (*depth*), encoded by chromosome 18 (Chr18).The total human proteome width is expanded taking into account modifications: by using different methods of calculation based on the average number of variations per gene from NextProt, a range of 0.55–7.14 million protein species in the human body was estimated. Chr18 encoded about up to 100 thousand of this number of proteoforms. The gene-centric investigation of proteome depth encoded by Chr18 was performed in the human blood plasma, liver tissue of healthy people and HepG2 cell line. We used panoramic analysis methods for cells – RNA sequencing and shotgun LC-MS/MS, along with the directional measurements for quantitative analysis. Information about the transcripts copy number per cell was obtained by qRT-PCR method, while the copy number of protein (corresponding to given transcripts) was obtained by targeted mass-spectrometry (SRM) with use of isotope-labeled peptides as standards. Comparing transcriptome *vs*. proteome could be used to confirm the robustness of the signals, detected by SRM. Taking Chr18 as an example, in liver tissue and HepG2 cell line there were confidently detected and measured transcripts and proteins for just 63% and 30% of 275 protein-coding genes, respectively. The results can be used for creation multi-channel test-systems for personal digital imaging in the field of molecular monitoring and health analytics. El.P., Ek.P. and Ek.I. acknowledge the Leading Scientific School of Prof. Andrey Lisitsa (№ NSh-6313.2018.4).

SpT.03-05
Structural basis of 14-3-3 protein-dependent interaction with human procaspase-2

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Caspase-2 (C2), a cysteine-dependent and aspartate-specific intracellular protease, has multiple roles in the DNA damage response, cell cycle regulation and tumor suppression. C2 functions as a central coordinator between the cell metabolism and apoptosis and its function is regulated by phosphorylation at several Ser residues. Phosphorylated procaspase-2 (proC2) binds to the 14-3-3 protein and this interaction blocks proC2 activation through an unknown mechanism. To elucidate this regulatory mechanism we showed that human procaspase-2 interaction with 14-3-3 is governed by phosphorylation at both S139 and S164. Next we combined the analytical ultracentrifugation, small angle x-ray scattering, chemical crosslinking, NMR and fluorescence spectroscopy to provide a structural description of proC2:14-3-3ζ complex and its binding interface. We showed that doubly phosphorylated procaspase-2 and 14-3-3 form an equimolar complex with a dissociation constant in the nanomolar range. We discovered that 14-3-3ζ protein interacts with proC2 predominantly through the N-terminal linker (123–153) and the N-terminal part of p12 subunit (372–381), thus stabilizing the structure of proC2 and protecting it from proteolytic degradation *in vitro*. Furthermore our analysis suggests that 14-3-3 protein binding reduces the flexibility of nuclear localization sequence (NLS) of proC2, given the diversity of proC2 subcellular localization, this might be yet another aspect how to influence the proC2 localization by masking NLS through phosphorylation and adaptor protein binding. Moreover, our results provide the first structural insight into the 14-3-3-dependent regulation of C2. This work was supported by the Czech Science Foundation (Project 17-00726S).

SpT.03-11
Interaction of transcription factor TEAD1 with its DNA response elements originating from human genes

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TEAD transcription factors mediate gene expression regulation through interactions with their DNA response M-CAT motif. They are active mainly during growth and development and induce gene expression encoding proteins involved in cell proliferation, differentiation or apoptosis prevention. TEADs and many of their target proteins are also known to be upregulated in several types of cancers. Thus TEADs are considered as a possible target for anti-cancer therapy. To study the interaction of DNA binding domain (DBD) of TEAD1 with 15 bp long M-CAT motifs originating from regulatory regions of human genes (namely *CTGF*, *SRF*, *C-MYC* and *GLUT1*), dissociation constant of each complex was determined using fluorescence anisotropy-based binding assay. Subsequently several structural mass spectrometry techniques, such as H/D exchange (HDX-MS), quantitative protein-protein chemical cross-linking and protein-DNA chemical cross-linking, were utilized to gain distance constrains and additional experimental data for structural

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characterization. According to K_D assay results, tested M-CATs could be divided into two groups: one with approximately ten times higher affinity to TEAD1-DBD than the other. HDX-MS revealed differences in deuterium uptake in helix H3, part of helix H2 and in the loop connecting them, identifying such region as DNA-binding pocket. This observation was also confirmed by quantitative chemical cross-linking as cross-link formation ability of helix H3 lysines (K57 and K88) significantly decreased in complex state. Minor protection from deuteration, probably caused by stabilization in complex state, was also observed at helix H1. Quantitative protein-protein chemical cross-linking resulted in 14 distance restrains that were, together with HDX-MS results, used for molecular docking to explain the structural basis of different affinities of TEAD1-DBD to each M-CAT element. Acknowledgments: CSF 16-24309S, MEYS/EU LH15010, LQ1604, LM2015043 CIISB, CZ.1.05/1.1.00/02.0109.

Monday 9 July
17:30–18:30, Panorama Hall

Biochemistry and signalling

SpT.02-06
Non-canonical binding of chloramphenicol and linezolid to ribosome revealed by MD simulations

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An interesting example of the dynamic behavior of ribosome nascent peptide exit tunnel (NPET) is its interaction with antibiotics, such as chloramphenicol (CLM) and linezolid (LZD). Modern conceptions about the mechanism of their action are based on X-ray diffraction analysis and claim that these antibiotics bound to the A-site of the peptidyl transferase center prevent binding of aminoacyl-tRNA. But biochemical experiments revealed that these antibiotics does not exclude peptidyl transferase reaction and selectively inhibits synthesis of certain peptide sequences (Marks et al., 2016), which contradicts accepted mechanism. Our simulation of CLM interaction with the *E. coli* ribosome in A,P-state suggests that CLM forms stacking interactions with U2506 and Ψ2504 bases, embedding itself between them, and hydrogen bonds with G2061 and Ψ2504 bases, while the dichloroacetyl moiety of CLM is oriented into NPET lumen, which favors its interaction with the nascent peptide. We obtained similar results for LZD: its morpholine moiety is embedded between U2506 and Ψ2504 bases, and its oxazolidinone ring forms hydrogen bonds with the G2061 base, being able to interact with the nascent peptide. CLM and LZD complexes structures modeled by us are in good agreement with the known biochemical data, allowing to explain selective inhibition of peptide synthesis. Noteworthy, U2506 and Ψ2504 nucleotides accept the conformation necessary for this interaction only during the translation elongation. Thus, the dynamic appearance of a binding site that exists only in a certain functional state is possible in the ribosome. This confirms the Koshland's principle of induced fit, according to which the ligand and the constitutively existing binding site mutually adjust their conformations in the process of interaction. All the calculations were performed with the Lomonosov supercomputer of Moscow State University using GROMACS 5 and PLUMED 2 packages and parm99sb force field.

SpT.02-10
Water tracking as an alternative method for proteins core exploration

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The increasing awareness of tunnels role in enzymes with buried active site pushed researcher to develop tools for pores, tunnels and pathways identification in macromolecules. The most recent can facilitate analysis of molecular dynamic simulations and gives information about the geometry of detected pathways and their behaviour during simulation. Generally they are constructing Voronoi diagrams to detect and describe voids within the macromolecule. By the use of defined probe radius and internal cavity location, user can detect tunnels providing access from the selected area to surrounding. Such approach possess several limitations and provides rather an approximation of tunnels to tubes with symmetrical diameter instead of real tunnel picture. Moreover the physicochemical properties of amino acids that builds tunnels are neglected. In principle, explicit solvent molecular dynamics simulations allow to study biological systems in a way to provide such information. Simulated protein is immersed in water box and during entire simulation water molecules are penetrating protein core. However, the identification and tracking of water molecules which enter regions important for catalysis requires screening of positions of thousands of single molecules for several thousand of molecular dynamics steps. To facilitate analysis of the behaviour of water (and if necessary other solvent molecules or ligands) we have developed AQUA-DUCT. Here we would like to provide an example of its usage for analysis of water transportation in selected enzymes (epoxide hydrolases and cytochrome p450). An advanced clustering and pathways trimming methods were used for ligand trajectories grouping. An instant and easy access to statistical data combined with visualisation of water entry clusters and each water molecule trajectory enable detection of transportation pathways, residues controlling access to active sites, preferable flow direction and other features not accessible by other tools.

SpT.02-13
Structural bioinformatics prediction of the conformational changes in ADAMTS13

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In most patients with acquired TTP, autoantibodies target an epitope in the spacer domain of ADAMTS13. An autoantibody resistant, gain-of-function variant (GoF) of ADAMTS13 was designed on the major epitope of spacer domain with conservative mutations. Structural studies revealed that GoF-ADAMTS13 exists in an open, while wild-type (WT) ADAMTS13 is found in closed conformation, with the C-terminal CUB domains bound to the spacer domain. Our aim was to employ modeling to identify the structural determinants in the CUB domains contributing to these conformational changes. The experimental structure of C-terminal CUB domains of ADAMTS13 is not available in Protein Data Bank. A homology model of the C-terminal CUB1-CUB2 domains was built. Domain interactions between the

spacer and CUB domains were studied by protein-protein docking where the structures were restrained between the major epitope residues in spacer domain and the predicted interface residues of CUB domains. GoF ADAMTS13 mutations were *in silico* introduced to final poses. Hence, both WT- and GoF-ADAMTS13 were subjected to binding free energy calculation on 100 ns molecular dynamics simulation trajectories. Subsequently, these poses were investigated to reveal which residues are contributing to conformational changes of ADAMTS13. A pose with relatively higher binding affinity against WT-spacer and lower binding affinity against GoF-spacer is found. The selected pose is in good agreement with the previously published experimental studies. Moreover, the pose is found to be informative to predict which residues are important for conformational changes. These residue predictions are subjected to *in vitro* mutation studies in order to test changes in conformation, proteolytic activity and resistance against autoantibodies. We have used the available structural bioinformatics tools to predict the nature of conformational changes which switch the human ADAMTS13 protein between open and closed conformations.

SpT.02-07
Reactivation of inhibited acetylcholinesterase with newly synthesized chlorinated pyridinium aldoximes

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Acetylcholinesterase (AChE) is an essential enzyme that hydrolyses the neurotransmitter acetylcholine (ACh) and is important in the control of neurotransmission in the neuromuscular and brain synapses. Organophosphorus (OP) compounds (i.e. nerve agents and pesticides) irreversibly inhibit AChE. Consequently, ACh accumulates in the synapses and overstimulates ACh receptors, which can result in a lethal outcome due to respiratory arrest. Therapy in OP poisoning comprises atropine (antimuscarinic drug), an oxime reactivator of OP-inhibited AChE, and an anticonvulsant drug. The standard oximes used in practice are not equally efficient in the reactivation of different OP-AChE conjugates. Moreover, they do not cross the blood-brain barrier (BBB) and thus, they do not reactivate brain AChE. Newly synthesized mono- and bis-chlorinated bispyridinium aldoximes were designed in order to keep some structural characteristics of previously reported non-chlorinated analogues that have been proven potent in the reactivation of inhibited AChE. An additional premise in their design was that the addition of chlorine atom would slightly increase the lipophilicity of these compounds in comparison to their analogues that cross the BBB at up to 6% of their blood concentration. Therefore, it is expected that these oximes would achieve higher concentration in the brain. The reactivation potency of the oximes was evaluated for AChE inhibited by various nerve agents (sarin, cyclosarin, VX, tabun) and pesticide analogue (paraoxon) and compared to analogous oximes. Although all six of the new oximes showed promising potency to reactivate OP-inhibited AChE, one oxime was proven as an extremely potent reactivator of AChE in case of cyclosarin, sarin and VX inhibition. Along with its predicted lipophilicity, this oxime is anticipated as both a peripherally and centrally active reactivator. This work was supported by the Croatian Science Foundation (4307) and Czech Grant Agency (18-01734S).

SpT.02-05
The cyclic AMP receptor protein (CRP) orchestrates the increase in intracellular levels of cAMP in response to osmotic stress in pathogenic mycobacteria

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The second messenger 3′,5′-cyclic adenosine monophosphate (3′,5′-cAMP) is involved in the adaptation of many bacteria, including mycobacteria, to the environment. Osmotic stress is encountered within the host but the link between cAMP and this particular stress in mycobacteria has not been explored in depth. Targeted metabolomic analysis was used to measure the intracellular levels of cAMP in mycobacterial strains after applying a stress with 250 mM sodium chloride, the concentration found within macrophages. Metabolites were extracted after 0, 6 and 24 h of stress by mechanical lysis. Samples were analysed using accurate-mass liquid chromatography mass spectrometry. We show that only the pathogenic *Mycobacterium tuberculosis* and *Mycobacterium bovis* BCG, but not the fish pathogen *Mycobacterium marinum* nor the non-pathogenic *Mycobacterium smegmatis*, respond to osmotic stress via a rapid *de novo* synthesis of cAMP. We further show that the increase in cAMP production depends on the cAMP receptor protein (CRP) but is independent of the Ser/Thr protein kinase PknD, which has previously been associated with the osmotic stress response in *M. tuberculosis*. It is established that cAMP intoxication subverts the host immune response in infected macrophages by pathogenic *M. tuberculosis*. In this study, we provide the first evidence that only in pathogenic mycobacteria is the CRP responsible for the production of high levels of cAMP in response to osmotic stress, conditions found to be in macrophages.

SpT.02-04
Study of lectins from *Photorhabdus luminescens* bacterium

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We present novel lectins from *Photorhabdus luminescens* with an unusual heptabladed β-propeller fold. These proteins show very high sequence identity with recently described lectins PLL and PHL from the Photorhabdus genus. Furthermore, all lectins are localized in a row in a bacterial genome. Lectins, a group of proteins capable of binding glycoconjugates specifically and reversibly, are showing their importance in both mutualistic and parasitic interactions between microorganism and hosts. Both of these stages are present in the *P. luminescens* life cycle. The bacterium is highly pathogenic towards the larval stadium of various insect species and mutualistic with infective juveniles of the nematode Heterorhabditis bacteriophora. For structural and functional characterization of Photorhabdus lectins, the wide range of methods was used, e.g. glycan array, surface plasmon resonance, isothermal titration calorimetry, analytical ultracentrifugation, X-ray crystallography and biological assays performed with human blood and hemolymph (reactive oxygen species production and phenoloxidase activity). Whereas heptabladed β-propeller fold is common for all studied lectins, the

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oligomeric state differs from mono- to di-mer. All studied lectins recognize L-fucose and O-methylated disaccharide glucose(1–4) rhamnose, but the fine binding specificities vary. The biological assay shows inhibition of the immune system response in the presence of *P. luminescens* lectins. Our results indicate these lectins might be involved in *P. luminescens* pathogenicity towards insect larva.

SpT.02-11
Effects of N-(3 oxododecanoyl)-L homoserine lactone, a quorum sensing signal molecule belonging to *Pseudomonas aeruginosa*, over human pancreas cell (hTERT-HPNE)

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Pseudomonas aeruginosa causes infections with high morbidity and mortality in patients with suppressed immune system. The mortality of these infections is directly related to the deterioration of membrane integrity of epithelial cells and inflammation formation. N-(3 oxododecanoyl)-L homoserine lactone (3-oxo-C_{12}-HSL) signal molecule which the bacteria have, play an important role in the arrangement of bacterial virulence factors and the formation of inflammation. In this study, it was aimed to determine the effects of 3-oxo-C_{12}-HSL on human pancreas cell (hTERT-HPNE). First, the cytotoxic effect of 3-oxo-C_{12}-HSL on hTERT-HPNE cell lines was detected with MTT. Second, the expression of cyclooxygenase-2 (Cox-2), a proinflammatory indicator in hTERT-HPNE cell stimulated with 3-oxo-C_{12}-HSL, on RNA and protein level was researched using real-time PCR (RT-PCR) and Western Blotting method. In the consequence of the performed MTT, it was found that 3-oxo-C_{12}-HSL signal molecule was cytotoxic on hTERT-HPNE (IC_{50} 75 μM). According to the results of RT-PCR, the expression level of *Cox-2* was increased significantly ($P < 0.05$) in the cell treated with 12.5-50 μM of 3-oxo-C_{12}-HSL when compared with control cell (%0.1 DMSO). The highest elevation was found to be fivefold at 50 μM concentration of 3-oxo-C_{12}-HSL. According to the results of Western Blotting analysis, the expression level of Cox-2 protein was increased significantly ($P < 0.05$) in the cell treated with 12.5-50 μM of 3-oxo-C_{12}-HSL when compared with control cell (%0.1 DMSO). The highest elevation was found to be 1.5 fold at 12.5 μM concentration of 3-oxo-C_{12}-HSL. These results suggest that 3-oxo-C_{12}-HSL signal molecule plays an important role in the increase of bacterial virulence, and also inflammation level in eukaryotic cells. This study was supported by the Anadolu University Research Foundation (Project Code: 1403F090).

SpT.02-08
Peculiarities of heme and iron metabolism in ticks

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Blood digestion in ticks is a key physiological process providing essential nutrients for development and fecundity of the parasite that ultimately allows transmission of tick-borne pathogens. Host blood is a rich source of pro-oxidative heme and iron. Therefore ticks had to evolve specific mechanisms protecting them against the oxidative stress caused by the surplus of these compounds. These adaptations led to unique features of tick heme and iron metabolism that markedly differ from other organisms including insect blood-feeders. In the majority of eukaryotic organisms, heme and iron homeostasis is based on balancing the flux between heme biosynthesis and its degradation. During evolution of their parasitic life style, ticks lost the capability of heme biosynthesis and all the heme needed for assembly their endogenous hemoproteins is of the host origin. Ticks are also not capable to acquire iron via heme degradation as they lack the enzyme heme oxygenase (HO). Our experiments confirmed, that iron needed for tick metabolic demands does not originate from digested hemoglobin but most likely from the serum transferrin. The intracellular free iron is maintained at low levels by its storage in ferritin 1 (Fer1) that is closely related to the mammalian heavy-chain ferritins, including the typically conserved motifs for ferroxidase center. Proteosynthesis of Fer1 is controlled at translational level via binding of iron-regulatory protein (IRP) to the iron binding element at 5′ UTR region of Fer1 mRNA. In contrast to their vertebrate hosts, ticks possess a specific type of secreted ferritin 2 (Fer2) that serves as inter-tissue transporter of bio-available iron, which resembles the function of vertebrate transferrin. These major departures of tick heme and iron metabolism and transport from their canonical functioning in their hosts hold promises for the design of effective anti-tick strategies. Financing: Czech Science Foundation No. 18-018132S (PK).

SpT.02-09
β-lactoglobulin covalent modification by phycocyanobilin under physiological conditions: structural and functional effects

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Phycocyanobilin (PCB) is a blue tetrapyrrole chromophore covalently attached to cysteine residues of C-phycocyanin (PC), chromoprotein of cyanobacteria *Spirulina platensis* and a molecule with numerous health-promoting effects. β-lactoglobulin (BLG) is the major protein of milk whey, frequently used as an additive in food industry, due to its extraordinary techno-functional properties. In this study, we investigated covalent binding of PCB to BLG under physiological conditions, using spectroscopic and electrophoretic techniques. We showed that BLG stereo-selectively binds PCB, with the apparent binding constant of 4×10^5 M^{-1}. Binding of PCB to BLG exhibits slow kinetics with binding rate constant (k_a) of 0.065 min^{-1}, while unfolding of BLG in urea makes free thiol group more exposed to solvent, producing a higher yield of adduct formation and accelerating the reaction ($k_a = 0.101$ min^{-1}). Although binding occurs at broad pH range, adduct formation rises with increasing reaction pH. Moreover, we demonstrated that covalent adduct could also be formed in simulated gastrointestinal conditions. In comparison to native BLG, phycocyanobilin-modified BLG has slightly altered secondary and tertiary protein structure. Obtained BLG-PCB adduct has increased antioxidant potential and is less susceptible to oligomerization and amyloid formation. Furthermore, BLG-PCB covalent adduct possess higher resistance to pepsin and pancreatin digestion then unmodified protein. Taken together, our results indicate that covalent modification of BLG with PCB could improve protein's bioactive and techno-functional properties and possibly enable incorporation of phycocyanobilin-modified protein into various food products.

SpT.02-12
Structural and polymorphic variability of *Bacillus subtilis* and *Bacillus cereus* TasA amyloid-like biofilm filaments

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Bacillus subtilis forms an extracellular biofilm matrix that is mainly composed of the protein TasA and an exopolysaccharide. Previous studies indicate that TasA forms amyloid-like filaments within the biofilm matrix, although the characteristic amyloid X-ray diffraction pattern has never been observed to confirm the presence of a cross-beta structure in TasA filaments. Here, we report the combination of solution NMR, solid-state NMR and X-ray diffraction to investigate the protein conformation in TasA filaments. TasA monomers are unstructured in solution, and X-ray diffraction analysis reveals a typical cross-beta stacking in the filamentous state, with additional reflections not characteristic for a cross-beta arrangement. Solid-state NMR line-widths indicate a high structural order of TasA molecules in the filaments, but intriguingly, chemical shift-based analysis shows that the TasA conformation in the filamentous state contains both beta-sheet and a-helical secondary structure elements. A comparison of solid-state NMR signatures of both Bacillus subtilis and Bacillus cereus TasA filaments reveals an only partially conserved fold of TasA with distinct a-helical propensity in the two species. Significant differences are observed for the structural polymorphism in B. subtilis and B. cereus filaments through solid-state NMR line broadening, suggesting a structural and dynamic variability during the TasA filament assembly process between different bacterial species.

SpT.02-02
Study of a low-CO_2-inducible protein in the marine centric diatom *Thalassiosira pseudonana*

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Diatoms are unicellular photosynthetic microalgae that live in almost all aquatic environments and their ecological importance relies on the ability to fix CO_2 and to use it as the building brick of other more complex organic molecules. As aquatic organisms, they have evolved mechanisms to cope with low CO_2 concentration, known as CO_2-concentrating-mechanisms (CCM) that help to improve CO_2 fixation by driving and concentrating CO_2 around Rubisco. The centric diatom, *Thalassiosira pseudonana,* was studied to decipher the responses to low CO_2 and a low-CO_2-inducible protein of 63 kDa (LCIP63) was found. Amino acid sequence of LCIP63 contains a domain from the NTF2-Like superfamily, which is repeated four times, and resembles a CaMKIII-association domain. In addition, LCIP63 contains a chloroplast signal peptide and is expressed in the chloroplast periphery as confirmed by electron microscopy. We hypothesize that LCIP63 could be involved in CCM as: (i) it is specifically induced by low CO2 concentration but not by other nutrients limitation, (ii) it shows carbonic anhydrase (CA) activity that is apparently dependent on the presence of a metal ion. Like many other CA secondary structure, that of LCIP63, analyzed by circular dichroism, shows high content of beta-sheets over alpha-helix, however no homology has been found to any known CA or to any other known protein using BLAST software. Some mutant strains using biolistic transformation have been produced and analyzed in order to better understand the physiological role of LCIP63 but to date more work is required to fully understand its function.

SpT.02-03
Structural understanding of nitrogen signaling in cyanobacteria via global nitrogen regulator NtcA and protein PipX

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Nitrogen assimilation is crucial for life. The CRP-family transcription factor NtcA is the global regulator of nitrogen homeostasis in cyanobacteria. Crystal structures of NtcA bound to its allosteric activator 2-oxoglutarate (2OG) and the potent NtcA coactivator protein PipX were determined (Llácer et al. PNAS 2010). PipX, a small monomeric protein composed of an all-β Tudor-like domain followed by two helices, mediates the control of NtcA function by the PII signalling protein: by sequestering PipX, PII renders PipX unavailable for NtcA activation. We determined the structures of NtcA bound to its target DNA and of the NtcA-PipX-DNA complex. The NtcA-DNA structure exemplifies the structural constancy of CRP type regulator-DNA complex, clarifying the mechanism of the NtcA specificity for its DNA box. We show that PipX activates NtcA by stabilizing the DNA binding-competent conformation of NtcA versus the multiple "inactive" NtcA forms also characterized by our group. Interestingly, while in the NtcA-PipX complex the C-terminal helix of PipX adopts a "flexed" conformation over the preceding helix, this was not the case in the PII-PipX complex, where the final helix was extended. This prompted us to investigate the structure of isolated PipX by NMR, showing that the C-terminal helix was "flexed". Thus, PII acts as an "opener" of the C-terminal helix of PipX, exposing a surface for potential ternary complex formation with other factors, as shown by interaction analysis with the transcriptional regulator PlmA (Labella et al., Front Microbiol 2016). Thus, as it stands, the PipX regulatory system involves two regulatory proteins, PII and PipX, and two transcriptional regulators, NtcA and PlmA. Evidence exists for more protein members of the PipX-regulon (Labella et al. Front Microbiol 2017). *Grants BFU2014-58229-P and BFU2017-84264-P and PrometeoII/2014/029 (Spanish & Valencian Governments) and ESRF synchrotron.*

Tuesday 10 July
17:30–18:30, Panorama Hall

DNA – genes – cells

SpT.01-09
Withdrawn

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SpT.01-11
Boundaries of Ultrabithorax gene regulatory domains

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Functional autonomy of domains in Bithorax complex (BX-C) of Drosophila is achieved through specialized insulators – boundaries. They ensure autonomy of each domain by blocking random contacts of promoter in one domain with cis-regulatory elements in other domains. However, boundaries also prevent interaction of promoters with tissue-specific enhancers located on the other side of the boundary. This paradox is explained by the fact that boundaries can interact specifically with promoters of target genes in the appropriate parasegments. Significant progress has been made in studying regulatory domains of Abd-B gene while regulation of Ubx remained poorly understood. We focused our efforts on studying boundaries of bx and bxd domains of Ubx. With the exception of Fab-7, all described boundaries in the BX-C have CTCF sites that are necessary for their function. Variable CTCF site (vCTCF) from third intron of Ubx was predicted to be a boundary. We deleted fragment that contains vCTCF and bxPRE. We observed weak bx phenotype caused by loss of strong PRE but not vCTCF. Thus we conclude that vCTCF is functionally unimportant. Another element for which a functional analysis has been undertaken is Fub-1. It's located between bx and bxd domains. It has CTCF site and is colocalized with CP190. Boundary function was ascribed to this element, however, we found that its deletion does not affect regulation of Ubx and does not result in gain of function phenotype. Strikingly, we found that Fub-1 is able to perform insulator function in attp50 platform where it replaces Fab-7. Taken together, this suggest that Fub-1 is redundant insulator, and in its absence another element act as a boundary. We propose model where Ubx promoter region act as an additional boundary. We are testing that hypothesis and continue functional analysis of Fub-1 and Fub-2 insulators and their bypass activity. This work was supported by Russian Scientific Foundation grant 16-14-10346.

SpT.01-05
Transcription complex assembly at the promoters of ribosomal protein genes

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Transcription regulation is a fundamental question, but some aspects of it remain unclear. Two types of promoters – housekeeping and developmental – have different control. In Drosophila different core transcription factors are necessary for housekeeping or developmental promoter function. Recently we described transcription factor and architectural protein – Opbp. Opbp binds to a very restricted set of sites (about 40 binding sites in the genome) located between promoter of ribosomal protein gene and developmental gene. We showed that Opbp is essential for viability, and loss of Opbp function, or destruction of its motif, leads to reduced ribosomal protein gene expression, indicating a functional role in promoter activation. Using yeast two-hybrid system, we showed that Opbp interacts with CP190 and TRF2. These proteins are essential for housekeeping gene promoter function. We assume that Opbp can attract CP190 and TRF2 to its binding sites. Using CRISPR/Cas9, we substituted *opbp* gene with an attP landing platform. Then we reinserted intact *opbp* gene and several variants with deletions of CP190 and TRF2 binding regions. This system allowed us to express mutated proteins at the wild-type background and at the same level. We will use these protein variants to study transcription complex assembly at the target promoters and changes in the expression of target genes. We also will use this system to examine the role of the Opbp dimerisation domain in local chromatin structure. We propose that Opbp-related ribosomal protein genes promoters can be a useful model for investigation of general regulation mechanisms of transcription. This study was supported by the Russian Foundation for Basic Research grant RFBR 16-04-01531.

SpT.01-06
Nuclear dynamics of the COMPASS subunit Spp1 prepares meiotic recombination sites for break formation

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Spp1 is the H3K4me3 reader subunit of the Set1 histone methylase complex (COMPASS/Set1C) that contributes to the mechanism by which meiotic DNA break sites (DSBs) are mechanistically selected. We previously proposed a model in which Spp1 interacts with H3K4me3 and the chromosome axis protein Mer2 that leads to meiotic DSB formation. Here we show that spatial interactions of Spp1 and Mer2 occur independently of Set1C. Spp1 exhibits dynamic chromatin binding features during meiosis with many *de novo* appearing and disappearing binding sites. Spp1 chromatin binding dynamics depends on its PHD finger and Mer2-interacting domain, and on modifiable histone residues (H3R2/K4). Remarkably, association of Spp1 with Mer2 axial sites reduces the *bona fide* turnover rate and diffusion coefficient of Spp1 upon chromatin binding, compared to other Set1C subunits. Our results indicate that 'chromosomal turnover rate' is a major molecular determinant of Spp1 function in the framework of meiotic chromatin structure that prepares recombination initiation sites for break formation.

SpT.01-07
Significance of poly(C)-binding proteins for pluripotent stem cells

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Pluripotent stem cells (PSCs) are characterized by their ability to self-renew and differentiate into all types of somatic cells. Oct4 is a key transcription factor of PSCs and is encoded by *Pou5f1* gene. This gene is regulated by its promoter, and also by distal (DE) and proximal (PE) enhancers. All of these elements are targets for methylation in differentiated cells and for regulatory proteins in PSCs. It was also shown that DE is active in naïve PSCs, and PE is active in primed PSCs. There are two essential elements present in the DE – site 2A and site 2B. Site 2B is a specific target for Oct4/Sox2 heterodimer, while the identity of protein (s) binding to the site 2A (CCCCTCCCCCC) has not been established; evidences suggest that these proteins are present not only in pluripotent, but also in differentiated cells. Using EMSA, affinity chromatography and mass-spectrometry approaches, we identified two members of poly(C)-binding proteins that can bind to the site 2A *in vitro* – hnRNPK and Pcbp1. We have confirmed the occupancy of the site 2A by hnRNPK in naïve PSCs by chromatin immunoprecipitation method, while Pcbp1 was detected at this site only in primed PSCs. Notably, the 1A site (GGGGGAGGGGTG) from the PE, which is similar to the site 2A present in the DE, shows a strong *in vivo* binding to the both hnRNP-K and Pcbp1 in primed PSCs. To carry out functional analysis we chose method of gene knock-out with CRISPR-Cas9 system for receiving of hnRNPK$^{-/-}$ and Pcbp1$^{-/-}$ PSCs. While hnRNPK-deficient naive PSCs lost their viability, Pcbp1-deficient naive PSCs were morphologically normal, except that they were relatively slow in growth rate. However, differentiation of Pcbp1-deficient naïve PSCs into primed PSCs was accompanied by massive cell death. Our data imply that hnRNPK and Pcbp1 are both critical for PSCs viability. The work was supported by the Russian Science Foundation (grant №17-14-01407).

SpT.01-03
Rapid and reversible formation of protein foci in response to hyperosmotic shock reveals possible bi-phasic architecture of the cytoplasm

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Current data show that the cytosol exhibits heterogenous distribution and diffusion of its components, and the basis of this heterogeneity is mostly unknown. However, cytoplasmic architecture should critically impact most cellular processes. We observed a novel phenomenon that highlights this architecture in yeast. During hyperosmotic shock, several GFP-labeled chaperones formed numerous Osmotic Shock Foci (OSF) which appeared and disappeared seconds after shock onset and removal. During shock the liquid phase of a cell is reduced in volume and concentrated, while putative solid structures, which cannot lose water, should come into contact, shaping the remaining liquid phase into foci-like areas. If we assume OSF-forming proteins to be trapped in the liquid phase, this model would explain the rapid appearance/disappearance of OSFs and also OSF protuberances and interconnections, which were observed using Structured Illumination Microscopy. Genome-wide microscopic screening revealed that only ~20 proteins, including chaperones, metabolic enzymes, P-body components and amyloidogenic proteins formed OSFs. Most OSF-forming proteins are known to form large assemblies. We discovered that OSF-forming ability of certain proteins was switchable in response to stress (for chaperones) and aggregation status (for amyloidogenic proteins). This provides a mechanistic explanation for how proteins can be trapped in the liquid phase – monomeric proteins or small complexes can travel through solid areas of the cytoplasm, while larger complexes cannot enter. In conclusion, our data suggest the cytoplasm, at least in yeast, may be more structured than previously thought and provide a model, based on the use of hyperosmotic shock, which allows study of some aspects of this structure. The study was supported by Russian Science Foundation grant 17-14- 01092. SIM microscopy was performed on equipment supported by the Program for Scientific Development of Moscow State University.

SpT.01-12
Dynamics of synaptic proteins in presynaptic boutons with single-molecule resolution

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Critical neuronal functions including neurotransmitter release and synaptogenesis are performed by dynamic macromolecular complexes at the synaptic membrane. Synaptic neuronal contacts are examples of complex structures, where proteins serve their function in a very confined and compartmentalized space. In order to decipher the dynamics and nanoscale organization of the CAZ (Cytomatrix of the Active Zone) and SNARE proteins

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in the presynaptic membrane by high-resolution microscopy, we grew hippocampal neurons on microstructured glass coverslips, functionalized with synaptic cell adhesion proteins. Formation of purely presynaptic sites is triggered on this microstructured host substrate, which we call "Xenapses". We suggest a new model of the presynaptic membrane compartmentalization: Bassoon forms the core of the active zone surrounded by synaptic vesicle fusion machinery; active zones are separated by endocytic zones. Another interesting finding is a spatial segregation of Syntaxin 1A and SNAP25B enriched domains in presynaptic membranes, which leads us to a conclusion, that these nanodomains work as buffers, accumulating SNARE proteins in different areas of presynaptic membrane, preventing uncontrolled synaptic vesicle fusion. Furthermore, the sptPALM analysis shows that these proteins are diffusionally rapped at nanodomains inside the presynaptic active zone. Moreover, CAZ-proteins are dynamic, disperse and sensitive to synaptic stimulation. Thus, Xenapses allow reconstructing separate membrane proteins nanodomains with a high localization precision providing novel information about the nano-organization of biological structures.

SpT.01-08
UBC gene transcriptional induction under arsenite-triggered cellular stress: unraveling the trans-acting factors

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The polyubiquitin gene UBC is considered a stress protective gene, upregulated under different stressful conditions, probably to meet the increased ubiquitin demand to remove damaged/misfolded proteins which are toxic to the cells. We previously identified, within the UBC promoter, the heat shock elements (HSEs), that were demonstrated to be responsible for the heat shock factor (HSF)1-driven induction of the UBC gene under MG132-induced proteotoxic stress. This study was undertaken to determine the molecular players driving the UBC gene transcriptional response to arsenite treatment, mainly addressing the role of the nuclear factor-erythroid 2-related factor 2 (Nrf2)-mediated antioxidant pathway. Exposure of HeLa cells to arsenite showed a time-dependent increase of UBC mRNA, while cell viability and, noteworthy, proteasome activity were not affected. Nuclear accumulation of both HSF1 and Nrf2 transcription factors was detected upon arsenite and MG132 treatments, while HSF2 nuclear levels only increased in MG132-treated cells. Notably, siRNA-mediated knockdown of Nrf2 did not affect UBC transcription under both basal and stressful conditions, while significantly impaired the constitutive and inducible expression of well-known antioxidant response element (ARE)-dependent genes. Consistently, the *in vivo* chromatin immunoprecipitation assay failed to detect Nrf2 binding to the UBC promoter sequence. By contrast, depletion of HSF1, but not HSF2, significantly compromised the stress-induced UBC gene expression. Significantly, HSF1-mediated UBC transactivation upon arsenite exposure relies on transcription factor binding to the distal HSEs previously mapped, and demonstrated to be involved in the UBC gene activation under proteasome inhibition. These data highlight HSF1 as the main stress sensor orchestrating UBC gene transcriptional induction by different stress signals.

SpT.01-01
N-TerPred: a new tool suite for the prediction of protein N-terminal modifications and organelle transit-peptide cleavage sites

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Proteins N-terminus and related modifications influence protein fate and half-life. The most frequent co-translational N-terminal protein modifications are linked to initial methionine excision and protein N-alpha-acetylation. Another modification occurring next to the protein N-term corresponds to the excision of the transit peptide of nuclear-encoded proteins targeted to the mitochondria and/or the plastids. Only few bioinformatics tools are currently available to predict mature proteins N-term such as Termi*N*ator3 (processing and modifications at position 1–2) and TargetP/ChloroP/SignalP tools that provide transit-peptide length predictions and protein subcellular localization. Unfortunately, the reliability of these tools suffer minor inconsistencies to major defects especially for the transit-peptide length prediction with the TargetP suite. Here, we present a dedicated database (*eNergiomeDB)* which data were collected from large scale N-terminomics studies. Experimental data related to protein N-terminus status and mature N-terminal start positions are complemented with subcellular localization information. *eNergiomeDB* provides the largest manually validated training set with almost 250,000 mature experimental N-termini related to more than 6,000 distinct non-redundant proteins (2,538 from *A. thaliana*, 3507 from *H. sapiens*). More than 175,000 validated N-terminus peptides (associated to 1,925 proteins) were used to improve the existing N-terminus prediction tools and to develop a new one. This newly developed N-terPred tool suite integrates a few new features including improved N-term acetylation prediction especially for NatC substrates, subcellular localization prediction based on protein primary sequence and reliable cleavage sites of plastid and/or mitochondrial transit peptide for the *A. thaliana* and human nuclear-encoded proteins. These newer functionalities and their possible use on alternative species will be presented.

SpT.01-10
Barley response to *Phytophthora* from a proteomics perspective

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Genus *Phytophthora* represents a world-wide spread pathogen with more than hundred recognized species and its devastating effect on plants has a serious economic and ecological impact. Recent studies found that *P. palmivora*, a well-known plague of tropical plants, can infect a non-natural host barley (*Hordeum vulgare*). This presents an interesting opportunity to employ a model crop plant and characterize molecular changes induced by *P. palmivora*. Barley seeds were surface-sterilized, germinated on a filter paper and transferred onto a liquid medium containing *P. palmivora* and components suitable for elicitins production. After 24 h, barley roots were collected and analyzed by *a gel-free* LC-MS and GC-MS for proteomics and metabolomics, respectively. The presence of *P. palmivora* in treated samples was confirmed

via a targeted SRM-based analysis of its proteins (even though there were no visible symptoms on seedlings phenotype) and PCA analyses of proteome/metabolome profiles clearly separated control plants from those incubated in the presence of *Phytophthora*. In total, we identified over 1,400 barley protein families and more than 140 showed a significant change in response to *P. palmivora* in three biological replicates. These candidates include proteins associated with the initial infection event, including jasmonate-responsive factors and mediators of ROS signalling. Furthermore, alterations in energetic metabolism correlate with the patterns found in metabolome profiling. This work was supported by grant LQ1601 of the Ministry of Education, Youth and Sports of the Czech Republic – project CEITEC 2020 and by the European Regional Development Fund, Project Phytophthora Research Centre Reg. No. CZ.02.1.01/0.0/0.0/15_003/0000453. Phytophthora strains for initial testing were obtained from the Collection of phytopathogenic oomycetes of RILOG (Ing. Mrázková).

Tuesday 10 July
17:30–18:30, Terrace 2A

Selected topics on education

SpT.04-01
Biochemistry in the knowledge-based society

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A knowledge-based society (KBS) is often described as a society with a strong capacity to create and utilize knowledge. Knowledge serves as a valuable asset of a KBS to promote its economy and social prosperity. In a KBS, education and research are highly valued; where there is a good number of scientists and engineers for research and development (R&D) and an educated labour force with specialized knowledge and skills. Information and communication technology (ICT) is also highly developed in a KBS; thus, people can conveniently use the internet, communicate and obtain information online. Taken together, education standard, scientist and engineer number, research output, and ICT infrastructure are the key indicators of a KBS. Hong Kong (HK) is currently transiting to a KBS. Although the ICT sector of HK is advanced, the investment in R&D in terms of the Gross Domestic Product (GDP) is still significantly lower than those of other Asian societies (Taiwan, Japan, and South Korea). For education, the figures of these societies are also higher than that of HK. We believe that R&D and education, especially the education of foundation science, such as biochemistry, should be a priority. To promote biochemistry education, we have conducted a preliminary study to survey three batches of students (Senior Secondary School students, University students in general, final year Life Sciences majors) in HK to learn about their understanding of KBS. The findings will be used to promote the education of biochemistry, for example, what kind of knowledge in biochemistry should be created in a KBS? And how the knowledge could be utilized? These are the fundamental questions we aimed at answering through our study.

SpT.04-02
Research integration of medical student: how can we manage this?

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Developments in molecular biology in last four decades, brought new terms into our terminology. For example genomics driven precision medicine is one of these points out to the importance of combining basic sciences into the clinics. This also requires more scientifically oriented investigator medical students that have learned the activity of scientific research in an early stage. So this changes also affected medical education basically means a reform in our curricula. With this perception a question rises, how can we manage integration of medical student to the research? In some cases mandatory research activities are seen or voluntary research programs included. Schools are offering mentorships for personal and professional development for the students. Also student led activities are in context. In papers there are obvious contributions of medical students in publication and these programs affected student's future career plans with increased interest in research itself and satisfaction. In this study we are focusing on finding answers to the question mentioned before with four main subtitles: (1) Benefits of research experience and restrictions in this way. (2) Mentorship with different aspects. (3) Research activity of medical student and its components in our Schools of Medicine, Istanbul University. (4) Proposing a model including three stages for the preparation of student into this process. Our expectation for this study: Reviewing the whole process and presenting our suggestions due to our previous study and experiences.

SpT.04-05
Teaching protein synthesis: development, evaluation and validation of a board game for use in different levels of teaching

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The relations amongst DNA, RNA, and proteins, by the complexity and high level of abstraction, can be facilitated by the use of diversified strategies. With this goal, a research was carried out that created and evaluated a rule-based game Synthesizing Proteins aimed at secondary and tertiary education, to assist in the teaching-learning process. The game content a picture board of a eukaryotic cell in section and is based on the use of cards representing biochemical processes and structures. After its development, the game was evaluated by 34 sciences teachers, as to its design, content′s approach and suitability of the use in classroom. After adaptations, it was investigated its contribution in the understanding of concepts and processes related to the main issue of 15 high school students. Finally, the application of the game in a regular classroom situation was evaluated, having the high school students acted as tutors. The game contribution was evaluated from the perspective of the interactions promoted (analyzed through the video records) and the conceptual understanding reached (analyzed through audio records collected during pre and post interviews). We found the students were able to identify the relationships among intracellular structures involved in proteins synthesis as well as the proteins' final targets. The interactions provided by the game influenced the students' learning process with the promotion of a meaningful, prospective learning.

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As a rule-based game, it also developed skills such as discipline, competition and strategic thinking. As for the use of the game within the classroom, some tutors highlighted positive aspects of the experience or suggested modifications on rules. The positive aspects indicated by them, in addition to the results of the present study, validate the use of the game in regular classes, with the collaboration of students-tutors. Free online version is in: http://eic.ifsc.usp.br/2014/01/15/sintetizando-proteinas/.

SpT.04-04
DNA-based analysis without laboratory equipment: *in silico* training of students on pharmacogenomics, genetic diagnostics, forensics or food analysis

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Analytical tests on genetic material are nowadays essential in many fields, such as diagnostics of disease, pharmacogenomics, identity testing, microbiological typing and detection of foodstuff adulteration. However, teaching laboratories often lack the resources to accommodate hands-on experimentation on this kind of analyses: samples, equipment, safety measures. There is hence a need for means to provide complete and up-to-date training for students in this field. The use of simulations and multimedia may fill such a gap, though it is particularly convenient that they are true spaces for experimenting, open-ended virtual laboratories rather than just animations or videos that always progress in the same way and end with the correct or expected result. We have developed a virtual laboratory that allows to perform experiments centred on the exploration of polymorphic regions of the genome. The techniques offered are fragmentation of DNA using restriction enzymes, PCR amplification, and electrophoretic separation. The system allows the users to design their own experiment and explore conditions, amounts, combinations... The results obtained are not prefabricated, but will depend on the actual conditions used. Such an open exploration may be very significant for assimilation of the underlying scientific concepts, both methodological and analytical/diagnostical, and to gain relevant professional abilities like experimental design, observation and analysis of results. We have used this tool in several scenarios: simulated paternity testing using STR markers from CODIS, with high school students; an RFLP analysis of beta globin gene and the sickle cell mutation, with BSc students; a multiplex PCR reaction for detecting alleles of CYP450 polymorphism, with Master students. More applications may be easily developed and applied in different teaching or training environments, since the virtual laboratory is a resource offered for free (biomodel.uah.es/en/lab/).

SpT.04-03
Biochemistry teaching – from natural history towards natural science

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As a discipline of the *Natural Science*, biochemistry is positioned in the "border area" between chemistry, biology and physiology. The "biological family" relationship is bringing some distinct features of the *Natural History* to our discipline, forcing it to be merely descriptive in many aspects. Whereas in "pure chemistry" the student may often – in principle at least – proceed along a line of logic and build-up on a limited number of pieces of background knowledge, for large areas of biochemistry there is only the possibility of *a posteriori* rationalization of the facts established by experimental research. The evolution followed rather erratic ways sometimes, and, if there is a rationale in the result, it is often well hidden. Quite often the Nature has chosen some improbable path, and we may just guess, why. This "descriptive" nature of biochemistry is probably among main causes why we encounter some disregard of biochemistry among our students, in particular among those who devoted themselves to theoretical chemical disciplines. (Our Department is responsible for teaching biochemistry to all chemistry students of our Faculty.) They – and unfortunately the best ones in particular – tend to a depreciatory attitude towards biochemistry. To counter this, the teacher has to put enough stress on the topics, which display most internal logic, and to place them towards the start of the lessons. In our opinion, suitable chapters in this respect are protein structure (where the physico-chemical principles play the decisive role) and energetic metabolism (which has a lot of own "chemical logic"). Although these topics obviously cannot represent a full coverage of the discipline, they may help to "sweeten" the burden of necessary and often boring memorization, as well as to attract the rationality-loving students to the still somewhat descriptive biochemistry and to motivate them to study of our discipline.

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